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Zaman et al., Soc. Neurosci. Abstr. 24, 471, 1998 with ~~PUB DATE~~

Crook et al., Nature Medicine 1998 April 4(4):452-5

Seabrook et al., Neuropharm. 1999 Jan. 38(1):1-17

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181.19

EXPRESSION OF ENDOGENOUS PRESENILIN 1 IN JURKAT CELLS. A. L. Schwarzman[1], N. Singh[1], M. Tsiper[1], L. Gregori[1]*, A. Dranovsky[1], M. P.Vitek[2], and D. Goldgaber[1]. [1]Dept. of Psychiatry, SUNY at Stony Brook, Stony Brook, NY 11794. [2]Dept. of Neurology, Duke University Medical Center, NC 27710.

In most tissues and cell cultures, the amount of presenilin 1(PS1) is extremely low and often not detectable by Western blot analysis or by immunofluorescence techniques. A constitutively high level of expression of endogenous presenilin was previously detected only in neurons. We showed now that PS1 is also highly expressed intracellularly and on the cell surface in Jurkat cells. Moreover, PS1 is concentrated at the surface of lamellipodia, and in particular, at the leading edge of lamellipodia in Jurkat cells adhered on a collagen matrix. Cell surface PS1 formed complexes with actin-binding protein filamin (ABP-280) which is known to mediate cell adhesion and cell motility. RANTES (8-kDa protein of cytokine family) up-regulated the expression of cell surface PS1 in dose-dependent manner. Our results suggest that PS1 represent a novel adhesion molecule in T cells.

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181.20

DEVELOPMENTALLY REGULATED EXPRESSI N OF PRESENILIN 1 IN HUMAN SH-SY5Y NEUROBLASTOMA CELLS C.Elle[1], D. Beher[1], S.E. Lichtenthaler*[1], C.L. Masters[2], K. Beyreuther[1] and G. Multhaup[1]; [1]ZMBH, University of Heidelberg, INF 282, 69120 Heidelberg, Germany; [2]Dept. of Pathology, University of Melbourne, Parkville, Victoria 3052, Australia

The majority of early-onset familial Alzheimer's disease (FAD) cases is caused by mutations in two related genes, the presenilin 1 (PS1) gene on chromosome 14 and the presenilin 2 (PS2) gene on chromosome 1. The expression of PS1 is supposed to be developmentally regulated and to play a role in neuronal development. Full-length PS1 is proteolytically processed into a ~30 kDa N-terminal fragment (NTF) and a ~20 kDa C-terminal fragment (CTF) by a so far unknown protease. In the rat brain and untransfected human SH-SY5Y neuroblastoma cells only the PS1-fragments but no full-length PS1 are detectable which indicates that the fragments might be the functionally active form of the protein. In primary hippocampal neurons of rat brain, PS1 is localized predominantly in the somatodendritic compartment mainly within the cell bodies and dendrites and to a small extent in axons. In the rat brain the PS1-fragments are associated with synaptic plasma membranes and colocalized in small synaptic vesicles. Thus, it is hypothesized that the N- and C-terminal fragments of PS1 might form a functional unit or play different roles in the cells while remaining associated. In order to investigate the effects of differentiation on PS1 expression in SH-SY5Y cells, untransfected as well as PS1-transfected SH-SY5Y cells were stimulated by nerve growth factor (NGF) and PS1 expression and processing were compared. The results suggest that the expression of PS1 and the production of NTF and CTF are developmentally regulated by cellular mechanisms which are NGF-mediated. In order to prove a potentially altered subcellular distribution of PS1 and the PS1-fragments in differentiated SH-SY5Y cells compared to undifferentiated SH-SY5Y cells and primary hippocampal neurons of rat brain, we investigated the PS1-distribution by sucrose density gradient centrifugation and immunoblotting analysis of the gradient fractions.

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181.21

DIFFERENTIAL EFFECTS OF TRUNCATION OF AMINO- AND CARBOXYL-TERMINAL DOMAINS OF PRESENILIN 2. T. Tomita*[1], A. Koyama[1], N. Takasugi[1], K. Aiba[1], S. Tokuhiro[1], T.C. Saido[2], K. Maruyama[3], T. Iwatsubo[1]. 1 Dept. of Neuropathol. & Neurosci., Univ. of Tokyo 2 RIKEN Brain Sci. Inst. 3 Natl. Inst. Phys. Sci., Japan

Mutations in presenilin (PS1 and PS2) genes cause early-onset familial Alzheimer's disease (FAD). PS is a polytopic integral membrane protein that spans membrane 8 times, with amino (N)- and carboxyl (C)- termini oriented to the cytoplasmic side. To learn about the roles of N- and C-terminal portions of PS2 for its functions, we constructed cDNAs encoding wild-type (wt) or N141I mutant (mt) PS2 truncated at the N- or C-terminal domains and examined the metabolism and stabilization of PS2 proteins as well as secretion of Aβ in COS or neuro2a (N2a) cells transfected with these genes. PS2 lacking the acidic stretch domain of the N-terminal 20 a.a. (PS2/dAS) was processed to form a shorter N-terminal fragment (NTF), which had a long half-life as revealed by cycloheximide treatment, whereas the whole PS2/dAS protein was short-lived. Mt PS2/dAS increased the production of Aβ42 at comparable levels to those with full-length (fl) mt PS2. In contrast, C-terminally truncated PS2 lacking the last 60 and 37 a.a. (PS2/388stop and PS2/411stop, respectively) were not processed to produce shorter CTF in N2a cells, and these proteins were short-lived. Notably, mt PS2/388stop or PS2/411stop did not increase the production of Aβ42, and the levels of secreted Aβ42 were at similar levels to those in cells expressing fl wt PS2. These results indicate that: (i) the C-terminal domain of PS2 is required for its stability, processing and Aβ42 promoting effects caused by mutation; (ii) the N-terminal acidic stretch domain is not necessary for its stability, processing and Aβ42 promoting effects; (iii) conditions under which nascent PS proteins are cleaved to produce NTF and CTF and form a stable complex may be the prerequisite for the normal and pathological functions of PS, in which the C-terminal domain of PS may play an important role.

DEGENERATIVE DISEASE: ALZHEIMER'S—PRESENILIN: MODEL SYSTEMS

182.1

THE EFFECTS OF PRESENILIN-1 MUTATIONS ON SYNAPTIC PHYSIOLOGY ASSESSED IN TRANSGENIC MOUSE MODELS S.H. Zaman*[1], A. Parent[1], A. Laskey[1], M.K. Lee[2], D.R. Borchelt[2], S. Sisodia[2], R. Malinow[1]; [1]Cold Spring Harbor Laboratory, CSH, NY, 11724; [2]John Hopkins University, Neuropathology Lab., School of Medicine, Baltimore, MD, 21205

Mutations in presenilin-1 are causative in ~50% of pedigrees of Familial Alzheimer's Disease, a condition with severe disruption of memory. The autosomal dominant mode of inheritance suggests a gain of function for the mutant forms of the protein. Transgenic mice overexpressing either the presenilin-1 A246E point mutation (PM) or the exon 9 deletion mutation (DM) were studied electrophysiologically. Brain slices prepared from the hippocampus were used to measure the amount of LTP, a cellular model of memory, and other synaptic electrophysiological parameters in PM, DL and wild-type (WT) mice. Synapses in the CA1 sub-field were stimulated by two independent pathways via the Schaffer-commissural fibers and monitored using an extra-cellular field recording electrode. Following tetanic stimulation, the amount of potentiation was generally greater in the mutants than in controls. For example, transmission from WT, PM and DM animals manifested 119±4.3 (N=9), 129±3.1(N=6) and 149±7% (N=6) of potentiation at 30 minutes after tetanic stimulation (DM > WT, $p<0.05$). Interestingly, in the presence of the GABA-A receptor blocker, picrotoxin, LTP was enhanced more in WT than in mutants such that the amount of LTP was not different among the three groups (WT:143±3.8, N=18; PM:145±3.2, N=18; DM: 152±3.2, N=13). These data suggest that mice expressing mutant PS-1 have a decrease in inhibitory tone, or aberration in other electrophysiological factors controlling the induction of LTP. It is possible that increasing inhibitory tone, as with benzodiazepines, could normalize LTP in mutant tissue and be beneficial to affected individuals. (Supported by Wellcome Trust, NINDS, Mathers Charitable Foundation).

182.2

BEHAVIORAL CHARACTERIZATION OF M146L MUTANT PRESENILIN 1 TRANSGENIC MICE. M.Gattu*, M.Grzelak, J. Naviera, & V.L.Coffin CNS/CV Biological Research, Schering-Plough Research Institute, Kenilworth, NJ 07033.

It has been well established that mutations in the presenilin(PS) 1 and presenilin 2 genes can cause early-onset familial Alzheimer's disease. Previous studies have shown that transgenic mice expressing the human mutant presenilin exhibit increased production of 1-42 beta amyloid protein in frontal cortex and hippocampus. To further explore the role of mutated presenilin genes in cognitive function, we examined transgenic mice expressing either wild type human PS1 or mutant human PS1 bearing the M146L mutation. Behavioral experiments in water maze showed no spatial memory impairment in M146L mutated PS1 transgenic mice up to 6 months of age compared to age-matched wild type transgenic or non-transgenic littermates. Similarly, no cognitive impairments were seen in 7 month old M146L mutated PS1 transgenic mice in a passive avoidance task when compared to non-transgenic littermates. Additionally, during the initial training period of an operant fixed ratio-discrimination task (working memory), 8 month old M146L PS1 transgenic mice did not differ in their performance when compared to age-matched wild type transgenic or non-transgenic littermates. Finally, administration of scopolamine, chlordiazepoxide and MK-801 revealed no difference in sensitivity to the disruptive effects of these agents between transgenic and non-transgenic mice working under this operant schedule. These data suggest that the presence of the M146L PS1 mutation does not produce cognitive impairments in mice of this age range.

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